β-Lactamases: quality and resistance

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β-Lactamase-mediated resistance to β-lactam antibiotics is a feature of great clinical significance. β-Lactamases are a diverse group of bacterial enzymes that vary in their abilities to hydrolyze β-lactam antibiotics. β-Lactamases possess an active site containing either a serine moiety or a zinc atom; serine β-lactamases are currently of greater clinical prevalence. This review considers the molecular classification of β-lactamases, the structure of the serine β-lactamase active site and the mechanisms by which β-lactamase production may become derepressed. The spread of β-lactamases in the clinical setting and some important structural mutations that have extended the hydrolysis profiles of serine β-lactamases are also discussed.

Key words: β-Lactamase, β-lactam, microbial drug resistance, molecular structure

INTRODUCTION

The number of β–lactamases that have been characterized has risen dramatically since these enzymes were first classified in 1973 [1]. Functional aspects of β-lactamase activity were reviewed in 1989 by Bush, who then proposed a system of classification [2–4]. A revised classification scheme published within the last year included more than double the number of different β-lactamases compared to the 1989 scheme [5].

The Bush–Jacoby–Medeiros classification is based on a functional assessment of each β-lactamase. It attempts to categorize these enzymes with respect to the efficiency with which they hydrolyze a range of different substrates and the extent to which they are inhibited by clavulanate [5]. Nevertheless, these different parameters show tremendous variability, and a simpler approach to β-lactamase classification, proposed by Ambler [6], involves consideration of the nucleotide sequences coding for these enzymes. This approach is considered further here, and is useful when considering the evolution of two particular classes of β-lactamase as defined by the molecular scheme: class A and class C. The relationship between this evolution and the clinical use of newer β-lactam antibiotics has been reviewed recently [7].

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MOLECULAR CLASSIFICATION OF β-LACTAMASES

There are two broad groups of β-lactamases: those that have a serine moiety at the active site and those that have an atom of zinc at the active site. The members of these two categories form the four classes of Ambler's molecular classification scheme [6]. Currently, serine-based enzymes account for three molecular classes and the zinc-based enzymes a single category. The two most prevalent and most important classes to date are the serine-based class C and class A enzymes.

The class A enzymes are primarily but not exclusively penicillinases and most are plasmid-determined [5]. The class C β-lactamases are also serine-based and are mainly chromosomally determined enzymes. They preferentially hydrolyze the cephalosporins and are present in almost all Gram-negative bacilli [8]. An exception to this is *Salmonella*, in which the chromosome lacks the structural gene for a chromosomal class C β-lactamase [9]. In many Gram-negative bacilli the class C β-lactamases are capable of conferring resistance. However, in others, such as *Escherichia coli*, their presence does not usually confer resistance [10]. Class D β-lactamases are a small group of serine-based enzymes evolutionarily related to the class C β-lactamases [11] and capable of rapid hydrolysis

of oxacillin [12]. Class B is composed of the zinc-based β-lactamases, which are very different structurally from the serine-based enzymes [13–15]. These are of increasing significance as many possess carbapenemase activity.

The majority of class C enzymes are inducible [5]. *E. coli* possesses *ampC*, *ampD* and *ampG*, three of the four genes that are necessary for induction, but lacks the fourth, the *ampR* gene [16,17]. The function of the β–lactamase in *E. coli* remains a mystery. The class A enzymes are constitutive in most species and inducible in a few species of lesser clinical significance [5]. There is now a great deal of information available concerning the tertiary structure of class A and class C enzymes. Examination of such data permits a greater appreciation of β-lactamase evolution.

β-LACTAMASES: THE HYDROLYTIC PROCESS

β-Lactamases are typically 100 times larger than their substrates: the enzymes have molecular masses of between 30 kDa and 40 kDa [5], whereas the β-lactams themselves have molecular masses in the region of 0.3 kDa.

β-Lactamases inactivate β-lactam antibiotics via disruption of the β-lactam ring [18]. The negatively charged oxygen of the carbonyl group on the β-lactam ring is attracted into the active site by the positively charged amine groups of serine-70 and alanine-237, resulting in the formation of an acyl–enzyme complex with the hydroxyl group of the serine moiety at the active site [19]. The following step, deacylation, involves the hydrolysis of the β-lactam ring. The presence of an appropriately oriented water molecule permits regeneration of the enzyme and the release of a hydrolyzed β-lactam that is microbiologically inactive [11].

This process involves a series of important binding interactions, including the binding of a series of hydroxyl groups with a variety of functional groups, usually amines, within this active site [19]. The β-lactam substrate has to be held in place for the active site serine to lyse this amide bond successfully. Therefore, the structure of the active site is of great importance.

STRUCTURE OF THE β-LACTAMASE ACTIVE SITE

The nucleotide sequences for all penicillin-binding proteins (PBPs) and all serine β-lactamases from both class A and class C contain common regions [20] (see Figure 1A). All possess a region (termed a motif) comprising a serine moiety, two other variable amino acids and a lysine moiety. Similarly, there are other motifs, the SDN motif and the KTG motif. These give rise to a series of hydrogen bonds between the positively charged amine groups and the negative hydroxyl groups.

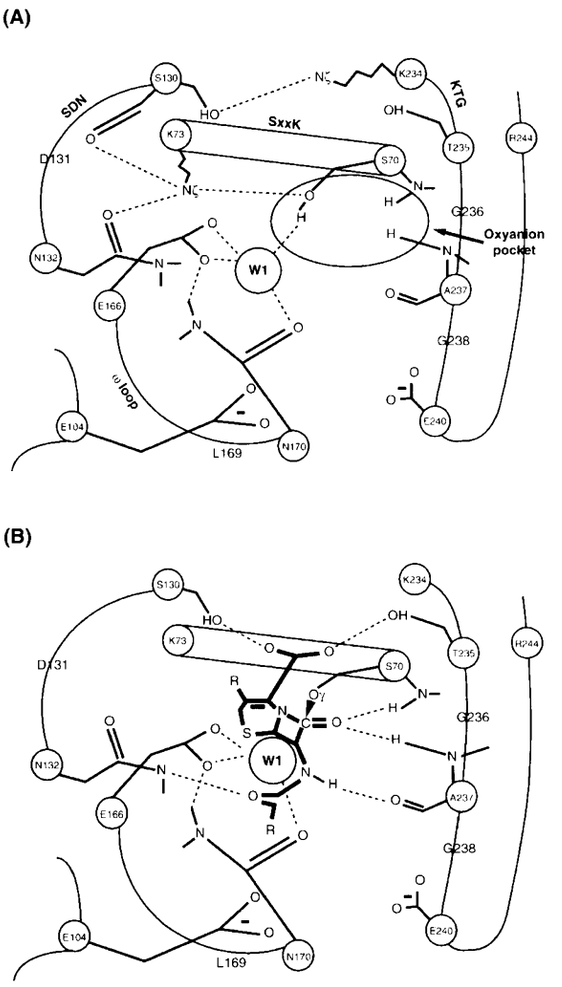

Figure 1 (A) Schematic showing the hydrogen bonding network and the amino acid residues of the binding site motifs of a class A β-lactamase. Note the importance of residues E166 and N170 of the omega loop in positioning a water molecule close to S70. **(B)** Hydrogen bond interactions between the functional groups of a cephalosporin molecule and the side-chains of the amino acid residues of the active site. Note that the NH groups of S70 and A237 hold the carbonyl of the β-lactam ring in the oxyanion pocket. The side-chains of S130 and T235 anchor one end of the molecule and those of N132 and A237 anchor the other. Adapted from Ghuysen [19]. Reprinted from Clinical Infectious Diseases, 24 (Suppl 1), Medeiros AA. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics, S19–45,

Crystallographic studies provide structural evidence for the nature of the β-lactamase active site. The TEM-1 β-lactamase molecule possesses an α-helical domain and a β-sheet of five antiparallel strands surrounded by α-helices [21]. At the N-terminus of the α-helix, H2 is serine-70 of the active site, in the numbering system of Sutcliffe [22]. Pivotal to the hydrolytic capabilities of the β-lactamase is the oxyanion pocket, located between the N-terminus of H2 and the B3 edge of the β-sheet. This region contains two positively charged amine groups. These groups interact with the carbonyl of the β-lactam ring and thus bind the antibiotic molecule, placing the amide bond of the β-lactam ring and the hydroxyl group of the active site serine in close juxtaposition.

Sections of the β-lactam molecule, apart from the carbonyl group of the β-lactam ring itself, play significant roles in the enzyme–substrate interaction [23]. A typical cephalosporin will be drawn to the active site of a TEM-1 β-lactamase by the powerful interaction between its negatively charged β-lactam carbonyl and the positively charged amines of the oxyanion pocket [21] (see Figure 1B). However, other significant binding interactions hold the substrate at the correct orientation within the active site. A carboxyl group (at the C4 position in cephalosporins), or an analogous substituent, is present in most β-lactam antibiotics, and forms hydrogen bonds with serine-130 and threonine-235 [24,25].

Another feature common to the β-lactam antibiotics, except the carbapenems and the clavams, is the peptide bond that holds the R-group (at the C7 position in cephalosporins). The positively charged amide and the negatively charged carbonyl enter into binding interactions with asparagine-132 and alanine-237, respectively [19].

These binding interactions permit the very precise positioning of the β-lactam in relation to the water molecule responsible for hydrolysis of the β-lactam-ring amide bond, thus making enzymes like the TEM-1 β-lactamase particularly efficient in hydrolyzing β-lactam antibiotics [19].

The omega loop

An important difference between the class A and class C β-lactamases is the presence of the omega loop in the class A enzymes. If a large β-lactam molecule with a rigid side-chain—such as ceftazidime or cefotaxime—attempts to fit into the active site of a TEM-1 β-lactamase, a portion of the structure known as the omega loop interferes with the orientation of the β-lactam's side-chain, preventing a precise fit. The omega loop is present in all class A β-lactamases, including the TEM-1 β-lactamase. It constricts the size of the active site cavity and provides a steric barrier to larger β-lactam molecules [21,26]. Therefore, these molecules are resistant to inactivation by the TEM-1 β-lactamase.

Aside from obstructing the entry of cephalosporins with large R-groups, the omega loop is significant because the hydrogen bonds formed by glutamate-166, a moiety within the loop, are instrumental in orienting the β-lactam-hydrolyzing water molecule, such that it is poised to hydrolyze or deacylate a β-lactam molecule as soon as an acyl–enzyme complex is formed [27].

The absence of this omega loop in class C β-lactamases makes the active site cavity much larger and much less efficient overall, certainly in hydrolyzing the penicillins [23]. However, this renders such enzymes capable of hydrolyzing larger β-lactams such as ceftazidime [21]. Furthermore, class C β-lactamases possess no residue that is functionally analogous to arginine-244 of the class A enzymes. The absence of arginine-244 alters the orientation of a water molecule that attacks the non-β-lactam ring of clavulanate or the penicillin sulfones. This renders class C enzymes non-susceptible to inhibition by these β-lactams [28].

β-LACTAMASES AND THE CLINICAL USE OF ANTIBIOTICS

All β-lactam antimicrobials are natural products or semisynthetic derivatives thereof. For this reason, the release of these compounds into the environment is partly responsible for the selection of many different types of β-lactamases. The clinical use of many different β-lactam derivatives has introduced a selective pressure promoting a corresponding diversification among β-lactamases in the hospital environment (Table 1) [7].

Until the early 1960s, penicillin G was the mainstay of β-lactam therapy, and correspondingly the only β-lactamase observed in the clinical situation was a staphylococcal penicillinase [29]. With the advent of penicillinase-resistant penicillins such as methicillin and broader-spectrum agents such as ampicillin, plasmids coding for β-lactamases spread throughout Gram-negative bacilli. These included TEM-1 and ultimately over 30 different plasmid-mediated β-lactamases [30]. Plasmids existed prior to the antibiotic era but did not appear to code for antibiotic resistance genes [31]. The presence of a selective pressure generated by the clinical use of antibiotics promoted the union of plasmids and resistance genes. This has led to the diversity of β-lactamases seen at present. Currently, the use of new β-lactams is perpetuating, indeed accelerating, this cycle [7].

Table 1 Stages in the history of β-lactamase development

Period	Antibiotics introduced	β-Lactamase response
1940–60	Penicillin	Staphylococci acquire β-lactamases
1960–78	Broad-spectrum penicillins and early cephalosporins	Plasmids determining β-lactamases (e.g. TEM-1) disseminate among Gram-negative bacilli
1978–95	Cephamycins, oxyiminocephalosporins, monobactams, carbapenems, β-lactamase inhibitors	1978—Promoter mutations cause *Klebsiella oxytoca* to hyperproduce its species-specific class A chromosomal β-lactamase
		1979—Regulator gene mutations cause enzyme hyperproduction in Enterobacteriaceae and *Pseudomonas* spp. that produce inducible chromosomal β-lactamase
		1982—Mutations of the structural genes of the plasmid-determined TEM, SHV and OXA β-lactamases enhance their affinity for third-generation cephalosporins and monobactams
		1982—*Enterobacter* and *Serratia* spp. acquire novel chromosomal genes that determine class A carbapenemases
		1988—Plasmids acquire genes that determine class C β-lactamases
		1988—Plasmids determining carbapenem-hydrolyzing metallo-β-lactamase emerge in Japan
		1991—TEM mutants have decreased affinity for clavulanate and sulfone β-lactamase inhibitors

β-Lactamase hyperproduction

In 1981, mutations in the β-lactamase induction-regulating genes led to the hyperproduction of class C β-lactamases by genera such as *Enterobacter*, *Citrobacter*, *Morganella* and *Serratia* [32]. Recently, Christine Jacobs, Jean-Marie Frère, and Steffan Normark elucidated the molecular mechanisms associated with the hyperproduction of class C chromosomal β-lactamases in Gram-negative bacteria [33] (Figure 2). Hyperproduction is associated with the processes of cell wall synthesis and cellular division [37,38]. Lysis of cell wall peptidoglycan leads to the formation of *N*-acetylglucosamine-anhydro-*N*-acetylmuramic acid-L-alanyl-D-glutamyl-*meso*-diaminopimelic acid (GlcNAc-anhMurNAc-tripeptide). The gene *ampG* codes for a permease which selectively permits the entry of this oligomer into the bacterial cell.

Inside the bacterial cell, an aminidase removes the GlcNAc residue, leaving the fragment anhMurNAc-tripeptide. An amidase encoded by the *ampD* gene then removes the tripeptide from the *N*-acetylmuramic acid. The tripeptide is recycled into new cell wall by linking to UDP-MurNAc to form UDP-MurNAc-tripeptide. In a final step, D-alanine-D-alanine is added, yielding UDP-MurNAc-pentapeptide, a murein precursor that is transported to the periplasm to be incorporated into new cell wall. This latter oligomer can bind to and modify a protein, a transcriptional activator (AmpR), that induces expression of the β-lactamase gene. When bound to the transcriptional activator, UDP-MurNAc-pentapeptide suppresses it, turning off production of AmpC β-lactamase (Figure 2A). anhMurNAc-tripeptide, the breakdown product of the peptidoglycan cell wall, can counteract the suppressive effect of UDP-MurNAc-pentapeptide. Increased cell wall breakdown due to β-lactam antibiotics or mutation of *ampD* leads to accumulation of anhMurNAc-tripeptide, which blocks the inactivator and triggers hyperproduction of AmpC β-lactamase (Figure 2B). Implied in this mechanism is a tremendous association between production of the AmpC β-lactamase and the regulation of cell wall formation, although it is unclear whether or not this β-lactamase has any direct function in terms of cell wall regulation. Hyperproduction of class C chromosomal β-lactamase is a phenomenon of clinical importance: 6% of patients with bacteremia due to *Enterobacter cloacae* and treated with a third-generation cephalosporin yielded a subsequent blood isolate

of the same strain, resistant to the antimicrobial used [39].

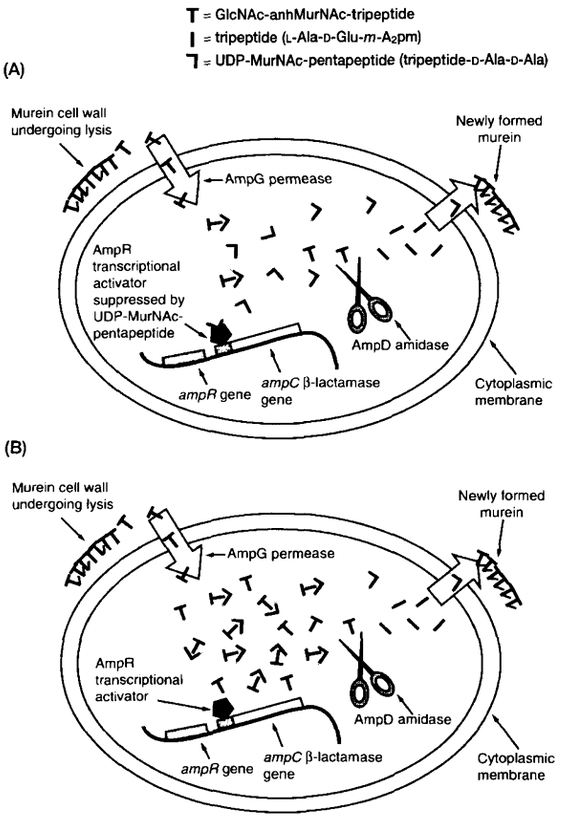

Figure 2 A model for peptidoglycan recycling and β-lactamase induction in enterobacteria. Hydrolases in the periplasm cleave the peptidoglycan cell wall to form the degradation product, *N*-acetylglucosamine-anhydro-*N*-acetylmuramic acid-L-alanyl-D-glutamyl-*meso*-diaminopimelic acid (**T**) [34]. AmpG permease transports it into the cytoplasm where an aminidase cleaves off the GlcNAc yielding anhydro-MurNAc-tripeptide. An amidase encoded by *ampD* recognizes specifically substrate containing anhydromuramic acid and cleaves the bond between it and L-alanine, releasing the stem tripeptide, L-alanine-D-glutamic acid-m-A_2pm (**|**). This tripeptide attaches to UDP-MurNAc which then accepts D-alanine-D-alanine to form UDP-MurNAc-pentapeptide (**⅂**). This murein precursor moves into the periplasm to form new peptidoglycan [33]. Under normal growth conditions, **⅂** also suppresses the AmpR transcriptional activator, probably by binding to it. **(A)** However, when large amounts of **T** exist in the cytosol, due to murein breakdown caused by β-lactams or mutation of *ampD*, the suppressive effect of **⅂** is nullified. **(B)** Thus, by regulating the relative amounts of **T** and **⅂**, *ampD* may control peptidoglycan composition [35,36]. The regulatory function of the AmpC β-lactamase, if any, is unknown. Adapted from [33].

Mutations of plasmid-determined β-lactamases

In 1983, mutations in the plasmid-borne genes for the SHV-1 β-lactamase led to an increased affinity for third-generation cephalosporins and monobactams [40]. These mutations have become widely disseminated in hospitals throughout the world, primarily in *Klebsiella* spp. Such strains were subsequently responsible for major outbreaks of β-lactam-resistant infection [41, 42].

At a molecular level in the TEM-1 β-lactamase, a single amino acid change from an arginine to a serine at the 164 position of the amino acid sequence has been shown to produce an extended-spectrum β-lactamase (ESBL), TEM-12 [21,26] (Figure 3A). This mutation reduces the steric hindrance of the omega loop with regard to the entry of third-generation cephalosporins such as ceftazidime. However, this is at the expense of hydrolytic efficiency; the omega loop is also instrumental in the accurate orientation of water within the active site [23]. Loss of rigidity in the omega loop compromises this aspect of its functionality, resulting in a weak ESBL that confers only low levels of resistance to third-generation β-lactams [43]. For these reasons, TEM-12 is of only minor clinical significance compared to some other ESBLs.

TEM-26, on the other hand, is an ESBL of greater efficiency. It has the same mutation as TEM-12 at position 164, reducing rigidity in the omega loop, but the glutamic acid at position 104 in TEM-1 and TEM-12 has been replaced with lysine (Figure 3B). This compensates for the loss in catalytic efficiency (arising from the reduction in rigidity of the omega loop) by enhancing the ability of TEM-26 to bind to its substrate.

Mutations within the TEM-1 binding cavity are of great clinical significance, but the TEM enzymes are by no means the only β-lactamase hierarchy capable of such modification. Similar mutations have been observed with OXA-1, PSE-2 and OXA-10 [44]. More recently, altered substrate specificity through mutation of the binding cavity in a class C β-lactamase was first described [45].

Following identification of this development, *Enterobacter* and *Serratia* with novel chromosomally mediated class A carbapenemases became evident in 1986 [46,47]. Fortunately, class A carbapenemases have remained relatively uncommon since then.

In 1988, plasmid-mediated class C β-lactamases similar to the chromosomal enzymes of *E. cloacae* and *Citrobacter freundii* were identified [48–50]. Some isolates from patients with *Klebsiella* infection were noted to possess a β-lactamase with a strong sequence homology to the *E. cloacae* class C β-lactamase. This conferred resistance to cefoxitin in addition to the

third-generation cephalosporins. There have been a number of reports of plasmid-mediated class C enzymes since 1988; some appear to be derived from *C. freundii*, others from *E. cloacae*, and some from *Pseudomonas* spp. [7].

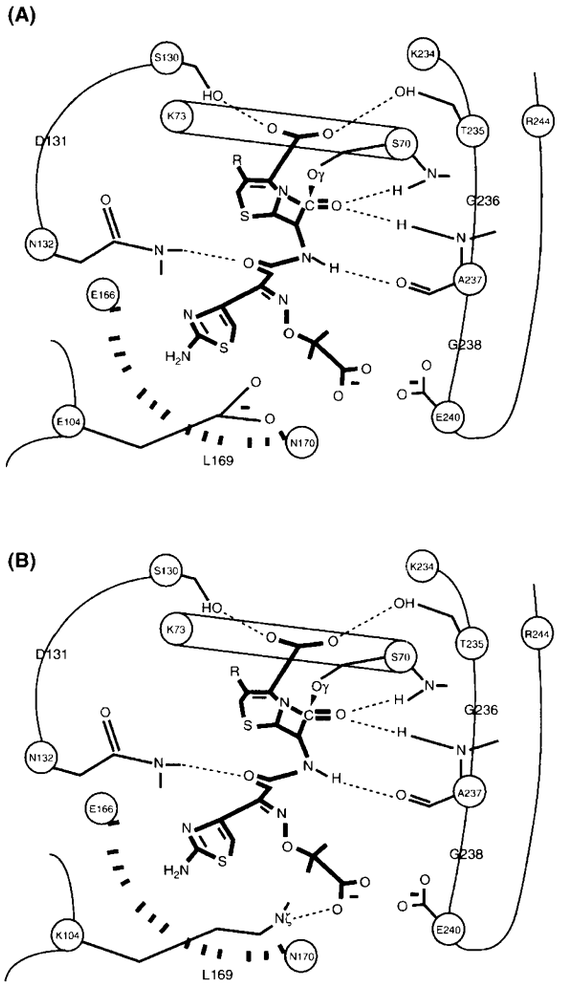

Figure 3 (A) Ceftazidime in the binding site of TEM-12 (R164S). The broken line between E166 and N170 represents a loosened, more flexible omega loop caused by the R164S mutation that weakens the bonding between R164 and N179 across the neck of the loop (not shown in this diagram). This provides more room for the bulky substituent of ceftazidime. Note that negatively charged side-chains of E104 and E240 repel the terminal carboxyl of the substituent. **(B)** Ceftazidime in the binding site of TEM-26 (R164S and E104K). The longer, more flexible side-chain of lysine bonds with the carboxyl moiety of the ceftazidime substituent, providing an additional anchor. Reprinted from Clinical Infectious Diseases, 24 (Suppl 1), Medeiros AA. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics, S19–45, © 1997 by University of Chicago.

During the early 1990s, TEM mutants occurred with greater resistance to inhibition by clavulanate and other β-lactamase inhibitors [51–53]. However, strains producing these enzymes are even more susceptible to first-generation cephalosporins than strains producing TEM-1, and are therefore unlikely to represent a major clinical threat.

In 1991, a plasmid-mediated class B β-lactamase capable of imipenem hydrolysis was reported from a single strain of *Pseudomonas aeruginosa* in Japan [54]. This was a cause for great concern, as carbapenemase activity is most uncommon in clinical practice. Then, in 1994, a *Serratia marcescens* isolate expressing a novel chromosomal class B carbapenemase (IMP-1) was described, again originating in Japan [55], and spread within several several hospitals [56]. In subsequent isolates of *S. marcescens*, the IMP-1 gene was carried by an integron-like element on a transferable large plasmid [57]. The IMP-1 β-lactamase gene has also been found in clinical isolates of *Alcaligenes xylosoxidans, Pseudomonas putida*, and *Klebsiella pneumoniae* [58]. This has the potential to be a major problem in Japan. A characteristic of Japanese clinical practice is the widespread use of imipenem/ cilastatin, and this may be implicated.

CONCLUSIONS

In summary, bacteria resist β-lactam antibiotics by several means. These include increased β-lactamase production, a modified rate of passage of antibiotics across the outer membrane, beneficial mutations in the binding cavity of an existing β-lactamase, and the recruitment of novel β-lactamase genes. The capacity to resist the onslaught of β-lactams in the clinic is possibly associated with the fact that bacteria have been exposed to a wide range of environmental β-lactams for many millions of years. Therefore, the selective processes necessary for resistance may have previously occurred in the environment and merely await extrapolation to the clinic. An example of this is the detection of a carbapenemase, IMI-1, in clinical isolates prior to the widespread clinical use of imipenem. For reasons such as this, the clinical importance of infectious disease remains undiminished. The continued search for new β-lactams is essential to sustain clinical success in the treatment of infection.

References

1. Richmond MH, Sykes RB. The beta-lactamases of gram-negative bacteria and their possible physiological role. Adv Microbiol Physiol 1973; 9: 31–88.
2. Bush K. Characterization of beta-lactamases. Antimicrob Agents Chemother 1989; 33: 259–63.

3. Bush K. Classification of beta-lactamases: groups 1, 2a, 2b, and 2b′. Antimicrob Agents Chemother 1989; 33: 264–70.
4. Bush K. Classification of beta-lactamases: groups 2c, 2d, 2e, 3, and 4. Antimicrob Agents Chemother 1989; 33: 271–6.
5. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 1995; 39: 1211–33.
6. Ambler RP. The structure of beta-lactamases. Philos Trans R Soc Lond B Biol Sci 1980; 289: 321–31.
7. Medeiros AA. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. Clin Infect Dis 1997; 24(suppl 1): S19–45.
8. Matthew M, Harris AM. Identification of beta-lactamases by analytical isoelectric focusing: correlation with bacterial taxonomy. J Gen Microbiol 1976; 94: 55–67.
9. Olsson O. Evolution of control sequences in enterobacterial *ampC* β-lactamase genes. Thesis. Umea, Sweden: University of Umea, 1983.
10. Normark S, Burman LG. Resistance of *Escherichia coli* to penicillins: fine-structure mapping and dominance of chromosomal beta-lactamase mutations. J Bacteriol 1977; 132: 1–7.
11. Galleni M, Lamotte-Brasseur J, Raquet X, et al. The enigmatic catalytic mechanism of active-site serine beta-lactamases. Biochem Pharmacol 1995; 49: 1171–8.
12. Dale JW, Godwin D, Mossakowska D, Stephenson P, Wall S. Sequence of the OXA2 beta-lactamase: comparison with other penicillin-reactive enzymes. FEBS Lett 1985; 191: 39–44.
13. Sutton BJ, Artymiuk PJ, Cordero-Borboa AE, Little C, Phillips DC, Waley SG. An X-ray-crystallographic study of beta-lactamase II from *Bacillus cereus* at 0.35 nm resolution. Biochem J 1987; 248: 181–8.
14. Concha NO, Rasmussen BA, Bush K, Herzberg O. Crystal structure of the wide-spectrum binuclear zinc β-lactamase from *Bacteroides fragilis*. Structure 1996; 4: 823–36.
15. Carfi A, Pares S, Duée E, et al. The 3-D structure of a zinc metallo-beta-lactamase from *Bacillus cereus* reveals a new type of protein fold. EMBO J 1995; 14: 4914–21.
16. Lindquist S, Galleni M, Lindberg F, Normark S. Signalling proteins in enterobacterial AmpC beta-lactamase regulation. Mol Microbiol 1989; 3: 1091–102.
17. Lindberg F, Lindquist S, Normark S. Inactivation of the ampD gene causes semiconstitutive overproduction of the inducible *Citrobacter freundii* beta-lactamase. J Bacteriol 1987; 169: 1923–8.
18. Waley SG. Beta-lactamase: mechanism of action. In Page MI, ed. The chemistry of beta-lactams. London: Chapman & Hall, 1992: 198–228.
19. Ghuysen JM. Molecular structures of penicillin-binding proteins and β-lactamases. Trends Microbiol 1994; 2: 372–80.
20. Ghuysen JM. Serine beta-lactamases and penicillin-binding proteins. Annu Rev Microbiol 1991; 45: 37–67.
21. Knox JR. Extended-spectrum and inhibitor-resistant TEM-type β-lactamases: mutations, specificity, and three-dimensional structure. Antimicrob Agents Chemother 1995; 39: 2593–601.
22. Sutcliffe JG. Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322. Proc Natl Acad Sci USA 1978; 75: 3737–41.
23. Matagne A, Frère J-M. Contribution of mutant analysis to the understanding of enzyme catalysis: the case of class A beta-lactamases. Biochim Biophys Acta 1995; 1246: 109–27.
24. Imtiaz U, Manavathu EK, Lerner SA, Mobashery S. Critical hydrogen bonding by serine 235 for cephalosporinase activity of TEM-1 beta-lactamase. Antimicrob Agents Chemother 1993; 37: 2438–42.
25. Dubus A, Wilkin JM, Raquet X, Normark S, Frère J-M. Catalytic mechanism of active-site serine beta-lactamases: role of the conserved hydroxy group of the Lys-Thr(Ser)-Gly triad. Biochem J 1994; 301: 485–94.
26. Maveyraud L, Saves I, Burlet-Schiltz O, et al. Structural basis of extended spectrum TEM beta-lactamases. Crystallographic, kinetic, and mass spectrometric investigations of enzyme mutants. J Biol Chem 1996; 271: 10482–9.
27. Herzberg O, Moult J. Bacterial resistance to beta-lactam antibiotics: crystal structure of beta-lactamase from *Staphylococcus aureus* PC1 at 2.5 Å resolution. Science 1987; 236: 694–701.
28. Delaire M, Labia R, Samama JP, Masson JM. Site-directed mutagenesis at the active site of *Escherichia coli* TEM-1 beta-lactamase. Suicide inhibitor-resistant mutants reveal the role of arginine-244 and methionine 69 in catalysis. J Biol Chem 1992; 267: 20600–6.
29. Neu HC. The crisis in antibiotic resistance. Science 1992; 257: 1064–73.
30. Medeiros AA. Plasmid-determined beta-lactamases. In Bryan LE, ed. Handbook of experimental pharmacology, Vol. 91. Berlin-Heidelberg: Springer-Verlag, 1989: 101–28.
31. Hughes VM, Datta N. Conjugative plasmids in bacteria of the 'pre-antibiotic' era. Nature 1983; 302: 725–6.
32. Sanders CC, Sanders WE Jr. Type I beta-lactamases of gram-negative bacteria: interactions with beta-lactam antibiotics. J Infect Dis 1986; 154: 792–800.
33. Jacobs C, Frère J-M, Normark S. Cytosolic intermediates for cell wall biosynthesis and degradation control inducible β-lactam resistance in Gram-negative bacteria. Cell 1997; 88: 823–32.
34. Holtje JV, Tuomanen EI. The murein hydrolases of *Escherichia coli*: properties, functions and impact on the course of infections in vivo. J Gen Microbiol 1991; 137: 441–54.
35. Normark S, Bartowsky E, Lindquist S, et al. The molecular basis of beta-lactamase induction in enterobacteria. In Neu HC, ed. New antibacterial strategies. New York: Churchill Livingstone, 1990: 161–75.
36. Tuomanen E, Lindquist S, Sande S, et al. Coordinate regulation of beta-lactamase induction and peptidoglycan composition by the *amp* operon. Science 1991; 251: 201–4.
37. Jacobs C, Huang L, Bartowsky E, Normark S, Park JT. Bacterial cell wall recycling provides cytosolic muropeptides as effectors for beta-lactamase induction. EMBO J 1994; 13: 4684–94.
38. Normark S. β-Lactamase induction in gram-negative bacteria is intimately linked to peptidoglycan recycling. Microb Drug Resist 1995; 1: 111–14.

39. Chow JW, Fine MJ, Shlaes DM, et al. Enterobacter bacteremia: clinical features and emergence of antibiotic resistance during therapy. Ann Intern Med 1991; 115: 585–90.
40. Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection 1983; 11: 315–17.
41. Bauernfeind A, Rosenthal E, Eberlein E, Holley M, Schweighart S. Spread of *Klebsiella pneumoniae* producing SHV-5 beta-lactamase among hospitalized patients. Infection 1993; 21: 18–22.
42. Sirot D. Extended-spectrum plasmid-mediated β-lactamases. J Antimicrob Chemother 1995; 36(suppl A): 19–34.
43. Jacoby GA, Medeiros AA. More extended-spectrum beta-lactamases. Antimicrob Agents Chemother 1991; 35: 1697–704.
44. Philippon A, Ben Redjeb S, Fournier G, Ben Hassen A. Epidemiology of extended spectrum beta-lactamases. Infection 1989; 17: 347–54.
45. Nukaga M, Haruta S, Tanimoto K, et al. Molecular evolution of a class C beta-lactamase extending its substrate specificity. J Biol Chem 1995; 270: 5729–35.
46. Medeiros AA, Hare RS. Beta-lactamase mediated resistance to penems and carbapenems among *Enterobacteriaceae* [abstract 116]. In: Program and abstracts of the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC. Washington, DC: American Society for Microbiology, 1986: 117.
47. Livermore DM. Carbapenemases: the next generation of beta-lactamases? ASM News 1993; 59: 129–35.
48. Bauernfeind A, Schweighart S, Dornbusch K, Giamarellou H. A transferable cephamycinase (CMY-ase) in *Klebsiella pneumoniae* (K. pn.) [abstract 190]. In: Program and abstracts of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, Georgia. Washington, DC: American Society for Microbiology, 1990.
49. Papanicolaou GA, Medeiros AA, Jacoby GA. Novel plasmid-mediated beta-lactamase (MIR-1) conferring resistance to oxyimino- and alphamethoxy-beta-lactams in clinical isolates of *Klebsiella pneumoniae*. Antimicrob Agents Chemother 1990; 34: 2200–9.
50. Rasmussen BA, Bush K, Keeney D, et al. Characterization of IMI-1 beta-lactamase, a class A carbapenem-hydrolyzing enzyme from *Enterobacter cloacae*. Antimicrob Agents Chemother 1996; 40: 2080–6.
51. Henquell C, Chanal C, Sirot D, Labia R, Sirot J. Molecular characterization of nine different types of mutants among 107 inhibitor-resistant TEM β-lactamases from clinical isolates of *Escherichia coli*. Antimicrob Agents Chemother 1995; 39: 427–30.
52. Henquell C, Sirot D, Chanal C, et al. Frequency of inhibitor-resistant TEM beta-lactamases in *Escherichia coli* isolates from urinary tract infections in France. J Antimicrob Chemother 1994; 34: 707–14.
53. Belaaouaj A, Lapoumeroulie C, Vedel G, Nevot P, Krishnamoorthy R, Paul G. Amino acid 241 of TEM-1 is highly critical in conferring resistance to ampicillin–clavulanic acid combination: molecular characterization of a natural mutant [abstract 944]. In: Program and abstracts of the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois. Washington, DC: American Society for Microbiology, 1991.
54. Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1991; 35: 147–51.
55. Osano E, Arakawa Y, Wacharotayankun R, et al. Molecular characterization of an enterobacterial metallo beta-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. Antimicrob Agents Chemother 1994; 38: 71–8.
56. Ito H, Arakawa Y, Ohsuka S, Wacharotayankun R, Kato N, Ohta M. Plasmid-mediated dissemination of the metallo-beta-lactamase gene bla_{IMP} among clinically isolated strains of *Serratia marcescens*. Antimicrob Agents Chemother 1995; 39: 824–9.
57. Arakawa Y, Murakami M, Suzuki K, et al. A novel integron-like element carrying the metallo-beta-lactamase gene bla_{IMP}. Antimicrob Agents Chemother 1995; 39: 1612–15.
58. Senda K, Arakawa Y, Ichiyama S, et al. PCR detection of metallo-β-lactamase gene (bla_{IMP}) in Gram-negative rods resistant to broad-spectrum β-lactams. J Clin Microbiol 1996; 34: 2909–13.